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CHEMICAL STIMULATION AND THE EVOLUTION OF CARBON DIOXID.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. XLIV.

EDWIN BINGHAM COPELAND.

[*Concluded from p. 98.*]

As HAS already been noted, the interpretation of my results with metallic poisons is uncertain. When the most of the experiments were performed it was without a suspicion that the CO_2 evolved from the plants could be anything but an immediate product of respiration. The results were presented in that light to the Botanists of the Central States at their meeting of December 1901. Since that time I have shown, and will present the evidence in the following tables, that these metallic salts can drive CO_2 from solutions of such salts as are in the cell sap. In any experiment, then, it may come directly from the proteid or from the sap, and it probably comes from both.

Before the experiment began each subject of experiment was left over night in its bottle, with a current of CO_2-free air passing through it *as rapidly as during the experiment.* The CO_2 evolved is expressed in cubic centimeters of normal acid for each hour. The data of the first experiment, except the temperature, are copied in full from my notebook, to illustrate the nature of the record. The poison was applied by opening the bottle and dropping in with a pipette enough of a concentrated solution to give the desired strength. The bottle is open very few seconds. I have satisfied myself repeatedly, by putting in water in the same way, that this introduces no error. The bottle was at once shaken thoroughly to insure the equal distribution of the poison. In each experiment except XXXVII Elodea was used.

The acceleration is rather great, considering the dilution; this is possibly due to the fact that the plants had been unusually long in the laboratory, and were respiring very slowly. The

TABLE II.

January 3. To test $\frac{M}{4000}ZnSO_4$ 100^{cc} baryta water = 12.32^{cc} $\frac{M}{100}$ acid.

TIME	EXPERIMENT		CONTROL	
	Titre	cc. N. per hour	Titre	cc. N. per hour
10:16–11:16	.....	.....	12.20	0.012
11:16–12:16	12.20	0.012	12.20	0.012
12:16– 1:52	12.15	0.011	12.18	0.009
1:52 $9.5^{cc}\frac{M}{100}$	$ZnSO_4$ put	into 370^{cc}in	exp. bottle,	making $\frac{M}{4000}$.
1:52– 3:04	12.05	0.022	12.20	0.010
3:04– 4:04	12.10	0.022	12.20	0.012
4:04– 5:10	12.10	0.c20	12.20	0.011
5:10– 7:10	11.90	0.021	12.12	0.010
7:10– 9:30	11.85	0.020	12.10	0.009
9:30–10:54 (13.4 hours)	10.05	0.017	10.85	0.011
10:54– 2:36	11.70	0.018	12.00	0.009
2:36 Control	given same	dose of	$ZnSO_4$.	
2:36– 5:00	11.80	0.023	11.85	0.020

great variation in the length of the intervals between the tests is a much more severe test of my method and apparatus than making observations regularly, say every hour or two hours, would be.

The next experiment shows a parallel use of double-distilled and tap water; the evolution of CO_2 is hastened slightly more from the latter.

TABLE III.

January 6. To test $\frac{M}{8000}ZnSO_4$. Double-distilled water in experiment bottle, tap water in control. Result in cc. N. per hour.

Time	Experiment	Control
9:33–10:39	0.015	0.020
10:39–11:51	0.022	0.022
11:51 $\frac{M}{8000}ZnSO_4$	in both bot	tles
11:51–1:51	0.024	0.034
1:51–4:51	0.025	0.036
4:51–7:45	0.025	0.032
7:45–9:47 (14 hours)	0.020	0.023

In the next experiment the difference between the tap water and the double-distilled water is doubtful. When it happened I ascribed the greater evolution of CO_2 from the former to imperfect distribution of the $ZnSO_4$.

TABLE IV.

January 10. To test $\frac{M}{16000}ZnSO_4$. Double-distilled water in experiment bottle, tap water in control. Results in cc. N. per hour.

Time	Experiment	Control
10:37–12:38	0.027	0.027
12:38– 2:08	0.027	0.027
2:08– 3:26	0.027	0.023
3:26 $\frac{M}{16000}ZnSO_4$	in both bot	tles.
3:26– 4:50	0.023	0.043
4:50– 5:50	0.027	0.035
5:50– 8:33	0.027	0.030
8:33–10:03	0.021	0.027
10:03–12:03 (14 hours)	0.028	0.028

The conclusion from this experiment is that $\frac{M}{16000}ZnSO_4$ exerts no appreciable stimulation.

We shall now take up the experiments with more concentrated $ZnSO_4$.

TABLE V.

December 30. Test of $\frac{M}{1143}ZnSO_4$.

Time	Experiment	Control
11:40–12:40	0.025	0.023
12:40– 1:46	0.027	0.027
1:46– 2:46	0.025	0.025
2:46 $\frac{M}{1143}$	$ZnSO_4$ in	exp. bottle.
2:46– 3:46	0.040	0.025
3:46– 4:46	0.038	0.025
4:46– 5:46	0.040	0.025
5:46– 7:46	0.035	0.025

In the remaining experiments the control will be omitted from the report, when it is a mere list of uniform figures.

TABLE VI.

December 17. Test of $\frac{M}{100}$ $ZnSO_4$.

Time	Experiment	Remarks
11 : 52–1 : 52	0.042	
1 : 52–2 : 52	0.040	
2 : 52 $\frac{M}{100}$	$ZnSO_4$ in	exp. bottle.
2 : 52–3 : 52	0.430	The water was poured from both bottles and boiled, and the CO_2 collected; the excess from the exp. bottle over that from the control was
	0.320	☜
Total CO_2 in first hour	0.750	A similar treatment another hour with fresh tap water showed
Total CO_2, second hour	0.665	☜
Total CO_2, third hour	0.550	

After the third hour the plants were put into water, and were alive but unsound the next morning.

TABLE VII.

December 18. Test of $\frac{M}{50}$ $ZnSO_4$.

Time	Experiment	Remarks
9 : 35–11 : 35	0.033	
11 : 35–12 : 35	0.030	
12 : 35– 2 : 53	0.033	
2 : 53 $\frac{M}{50}$	$ZnSO_4$ in	exp. bottle.
2 : 53– 3 : 53	0.490	The CO_2 was driven off as in the last exp.
Excess from exp.	0.300	
Total CO_2	0.790	

The preceding experiments were all performed at 19°–21° C. The temperature for the next one was 28°–29° C.

TABLE VIII.

December 16. Test of $\frac{M}{50}$ $ZnSO_4$.

Time	Experiment	Remarks
9 : 36–11 : 42	0.045	
11 : 42– 1 : 42	0.045	
1 : 42 $\frac{M}{50}$	$ZnSO_4$ in	exp. bottle.
1 : 42– 2 : 42	1.000	The CO_2 driven from both bottles.
Excess CO_2 from exp.	0.160	
Total CO_2	1.160	

In the experiments with $\frac{M}{50}$, $\frac{M}{100}$, and $\frac{M}{200}$ it was 10–15 minutes before the precipitation of $BaCO_3$ was apparent; then it became very rapid at once.

The bare end-results of some experiments at higher temperatures will be sufficient.

TABLE IX.

December 13. Test of $\frac{M}{100}$ $ZnSO_4$ at 37.5° C.

Time	Experiment	Remarks
2 : 12–3 : 12	0.060	
3 : 12 $\frac{M}{100}$	$ZnSO_4$ in	exp. bottle.
3 : 12–4 : 12	0.800	Including excess CO_2 from exp. bottle
Total CO_2	0.925	☜

The plants were put into water and remained alive.

TABLE X.

December 12. Test of $\frac{M}{200}$ $ZnSO_4$ at 37° C.

Time	Experiment
10 : 05–2 : 11	0.088
2 : 11 $\frac{M}{200}$	$ZnSO_4$ in exp. bottle.
2 : 11–3 : 11	0.815
Total CO_2	1.315

TABLE XI.

December 9. Test of $\frac{M}{500}$ $ZnSO_4$ at 39.5° C.

Time	Experiment
2 : 05–4 : 05	0.087
4 : 05 $\frac{M}{500}$	$ZnSO_4$ in exp. bottle.
4 : 05–5 : 00	0.743
5 : 00–6 : 30	0.627

TABLE XII.

December 4. Test of $\frac{M}{1000}$ $ZnSO_4$ at 36° C.

Time	Experiment
3 : 41– 5 : 32	0.109
8 : 10 $\frac{M}{1000}$	$ZnSO_4$ in exp. bottle.
8 : 10–10 : 10	0.207

TABLE XIII.

Temp.	Dilution	Increment
28°C.	50	2480
19	50	2290
19	100	1775
37.5	100	1440
37	200	1390
39.5	500	800
36	1000	90
19	1143	60
19	4000	100
19	8000	30
19	16000	0

If the results of these experiments with zinc are roughly summed up for comparison, it will be seen that the high acceleration by $\frac{M}{4000}$ presents the only exception to the rule that the more zinc applied, the more CO_2 is driven off. The figures here are the per cent. increment of CO_2 evolved after poisoning. The numerator (M) of the fractions is omitted.

The experiments on the influence of copper will be taken up next. The temperature in all of these was in the neighborhood of 20° C.

TABLE XIV.

January 20. Test of $\frac{M}{80000}$ $CuSO_4$.

Time	Experiment	Control
10 : 08–3 : 55	0.030	
3 : 55 $\frac{M}{80000}$	$CuSO_4$ in exp.	bottle.
3 : 55–5 : 01	0.030	0.030
5 : 01–7 : 16	0.027	0.027
7 : 35 Same	dose given to	control.
7 : 35–8 : 36	0.031	0.030

$\frac{M}{80000}$ $CuSO_4$ is then without demonstrable influence on the respiration.

TABLE XV.

January 15. Test of $\frac{M}{40000}$ $CuSO_4$.

Time	Experiment
8:45–10:27	0.029
10:27–12:27	0.032
12-27– 2:15	0.027
2:15 $\frac{M}{40000}$ $CuSO_4$	in exp. bottle
2:15– 3:15	0.039
3:15– 5:27	0.049
5:27–11:27	0.051
11:27–10:32	0.044
10:32– 5:02	0.042
5:02– 9:38	0.044

TABLE XVI.

January 13. Test of $\frac{M}{20000}$ $CuSO_4$.

Time	Experiment
11:46–1:16	0.041
1:16–2:28	0.042
2:28–3:46	0.043
3:46–4:52	0.042
4:52 $\frac{M}{20000}$ $CuSO_4$	in exp. bottle.
4:52–5:52	0.073
5:52–7:52	0.067
7:52–9:52	0.072
9:52–9:52(12hrs.)	0.062

The record in table XV is given for the entire period of observation. It should be said that from most of the tables both ends have been omitted, the published record covering the time more immediately before and after poisoning.

Table XVI shows less CO_2 was evolved toward the end of the experiment, but the material remained alive.

The plants were left in water over night; but few cells were alive the next morning.

TABLE XVII.

December 19. Test of $\frac{M}{10000}$ $CuSO_4$.

Time	Experiment
11:38–2:44	0.034
2:44 $\frac{M}{10000}$ $CuSO_4$	in exp. bottle.
2:44–3:44	0.100
Exc. CO_2 in water of exp. bottle	0.010
Total CO_2	0.110

TABLE XVIII.

December 20. Test of $\frac{M}{5000}$ and $\frac{M}{2000}$ $CuSO_4$, there being no control.

Time	Experiment A	Experiment B
10:00–2:12	0.040	0.038
2:12 $\frac{M}{5000}$ $CuSO_4$	in exp. A bottle.	$\frac{M}{2000}$ in exp. B bottle.
2:12–3:12	0.275	0.335
Total CO_2	0.320	0.435

The CO_2 was driven from both bottles, and the estimated content at the beginning of the hour subtracted; the remainder added to that found by titration.

Both bottles were treated in the same way another hour, with practically the same result. After the first hour most cells were alive in both bottles; but after two hours the plants of exp. B were practically dead.

The result of this experiment made it appear exceedingly probable that CO_2 was evolved after death under the influence of copper. The next two experiments show that this is the case.

TABLE XIX.

December 26. Test of $\frac{M}{2000}$ $CuSO_4$.

Plants boiled for twenty minutes in distilled water; then cooled and washed until no CO_2 came off.

Time	Experiment
3:39–4:51	0.000
4:51 $\frac{M}{2000}$ $CuSO_4$	in bottle.
4:51–5:57	0.082
5:57–7:39	0.029
4:51–7:39 (total CO_2)	0.140

TABLE XX.

January 9. Test of $\frac{M}{1167}$ $CuSO_4$.

This material was boiled twice in double-distilled water.

Time	Experiment
5:27–6:27	0.007
6:27 $\frac{M}{1167}$ $CuSO_4$	in exp. bottle.
6:27–7:33	0.429

This raised the question as to whether or not Zn might act in the same way on boiled Elodea. Experiment shows that it does.

These results were construed as proving that dead "protoplasm" could be caused to evolve CO_2 by the action of Cu and Zn. As a matter of curiosity I made a similar experiment, using $\frac{M}{1000}$ $CuSO_4$ on peptone (in distilled water); no CO_2, or at most a trace, was evolved.

TABLE XXI.

December 28. Test of $\frac{M}{100}$ $ZnSO_4$. The material was boiled under suction, and washed until no CO_2 came off.

Time	Experiment
8:53–9:53	0.000
10:04 $\frac{M}{100}$ $ZnSO_4$	in bottle.
10:04–11:04	0.020
11:04–12:22 (12.3 hrs.)	0.004
Total CO_2 evolved	0.070

The results of the experiments with copper on live plants are summarized here, in the form explained in connection with the similar table on zinc:

TABLE XXII.

Dilution	Acceleration, Per Cent	Dilution	Acceleration, Per Cent
2000	1045	20000	52
5000	700	40000	47
10000	224	80000	0

The next table shows the action of several concentrations of mercury.

TABLE XXIII.

January 23. Tests of $HgCl_2$.

Time	Experiment	Control
10:28-2:04	0.029	0.033
2:04 $\frac{M}{160000}$	$HgCl_2$ in exp. b	ottle.
2:04-7:46	0.035	0.027
7:46 $\frac{M}{80000}$	$HgCl_2$ in control	bottle.
7:46-11:34	0.037	0.039
11:34-11:16 (11.7 hrs.)	0.027	0.032
11:16 A. M. Exp. alive	and sound. Co unsound.	ntrol mostly alive,
1:58 P. M. $\frac{M}{10000}$	$HgCl_2$ in exp. bottle.	$\frac{M}{1000}$ in control bottle.
1:58-2:58	0.235	0.685
2:58 Exp. culture	practically dead.	Control dead.

The solutions were poured off to determine the CO_2 in them, and to investigate the post-mortal "respiration" similar solutions made up with tap water were put on the dead Elodea in the bottles. This was followed by practically the same evolution of CO_2 as when the solutions were first applied; which seemed to show that the substance from which the CO_2 was driven off was not exhausted enough to interfere with the reaction. In this connection it was assumed that the evolution of CO_2 gradually slackened under the influence of an individual dose of poison because the mercury entered into combination and ceased to act. The material was then devoted to a determination of how much CO_2 might be driven from it by an indefinitely repeated renewal of the dose of $HgCl_2$. When this had gone on long enough to become monotonous, I varied it by applying the same amount of $HgCl_2$ in less water, making it more concentrated. *Less* CO_2 was evolved. I then put 700^{cc} of tap water, without Elodea, in each bottle, and gave it the dose which the Elodea and water had been receiving, with the same result. The $HgCl_2$ applied was:

Exp. bottle, $0.7^{cc}\,\frac{M}{10}$ Control, $7.0^{cc}\,\frac{M}{10}$.

Total CO_2 evolved in 24 hrs. - 0.535^{cc} N. - 2.370^{cc} Normal.
The CO_2 which can be washed from seven hundred cubic centimeters of tap (lake) water varies, but is less than 0.310^{cc} N. - - 0.310^{cc} N., leaving as result of the $HgCl_2$ at least - 0.225^{cc} N. - 2.060^{cc} N., which is more in both cases than the equivalent of the $HgCl_2$ applied.

Evidently no conclusions can be drawn as to the respiration of Elodea, dead or alive, from work with Hg in the presence of tap water. I therefore washed a lot of Elodea very thoroughly in double distilled water for the next experiment.

TABLE XXIV.

March 7. Tests of $HgCl_2$.

Time	Experiment	Control
11:09–3:39	0.070	0.059
3:39 $\frac{M}{80000}$	$HgCl_2$ in exp.	bottle.
3:39–4:39	0.095	0.058
4:39–6:33	0.076	0.059
6:33 $\frac{M}{1000}$ $HgCl_2$ in exp. run through wa	bottle. Plants re ter to wash it.	moved from control, and air
6:33–10:24 (15.85 hrs.)	0.530	(Total CO_2), 0.110^{cc} N
10:24–11:37	.....	(Total) 0.012
11:38 $\frac{M}{80000}$	$HgCl_2$ in con	trol bottle.
11:38–12:40	.....	(Total) 0.060
12:40 $\frac{M}{1000}$	$HgCl_2$ in con	trol bottle.
12:40– 8:00		(Total) 0.160^{cc} N

The last part of this experiment, with the water in which the control plants had stood, shows that CO_2 is driven from substances, presumably carbonates, as of lime, passed from the plants into the water. The evolution of CO_2 caused by $\frac{M}{1000}$ $HgCl_2$ was less excessive than when the plants were present; this I do not ascribe to a reaction with the proteid, but rather to the presence of the cell sap. The difference is doubtless because of the limited amount of carbonates which had passed from the plants to the water outside. The experiment as a whole shows that no necessarily valid conclusions can be drawn as to the stimulation of the respiration of Elodea with $HgCl_2$, even using the purest distilled water.

The significance of the observations on the influence of Zn and Cu was immediately in doubt. Acting on the suggestion derived from the similar effect of $HgCl_2$ on tap water and on distilled water after the Elodea had stood in it, I used tap water with Zn and Cu, so that the reaction, if any, should be more considerable.

TABLE XXV.

March 9. Action of Zn and Cu on tap water.

Time	Experiment	Control
12 : 23–1 : 23	0.005	0.010
1 : 23 $\frac{M}{1000}$ $ZnSO_4$ in exp.	bottle. $\frac{M}{10000}$	$CuSO_4$ in con.
1 : 23–2 : 25	0.150	0.052
2 : 25 Exp. stopped. $\frac{M}{1000}$	$CuSO_4$ in con	trol bottle.
2 : 25–3 : 25		0.645

These results approach what I got with the same "stimulants" with Elodea in the water.

A test of the apparent action of $AgNO_3$ on the respiration was followed immediately by one with tap water.

TABLE XXVI.

May 1. Test of $AgNO_3$.

Time	Experiment
8 : 44–1 : 45	0.042
1 : 45 $\frac{M}{40000}$ $AgNO_3$ in	exp. bottle.
1 : 45–2 : 45	0.130
2 : 45–3 : 45	0.095
Tap water then used, with	out any plants.
5 : 34–7 : 04	0.037
7 : 04 $\frac{N}{10000}$ $AgNO_3$	in bottle
7 : 04–8 : 04	0.080

This is just near enough to the result with Elodea to rob the latter result of significance as to the respiration.

In another test of the action of $AgNO_3$ on tap water, the water was boiled and cooled with a stream of CO_2-free air passing through it. It was poured into another bottle and then dosed with $AgNO_3$, which drove off only a trace of CO_2. The difference between this and previous experiments with boiled water must have been that the precipitated carbonate was gotten rid of by changing bottles.

$NiCl_2$ was tested similarly to $AgNO_3$.

TABLE XXVII.

May 13. Test of $NiCl_2$. The experiment bottle contains Elodea in 400^{cc} "Neptune" (a triple-distilled commercial water). The control bottle contains 400^{cc} tap water.

Time	Experiment	Control
11:05–12:59	0.027	0.003
12:59 $\frac{M}{1000}$	$NiCl_2$ in both	bottles.
12:59– 2:59	0.035	0.026
2:59– 6:23	0.034	0.018

The acceleration during the first two hours is decidedly more considerable with the tap water than with the Elodea. The rather rapid decrease, while the Elodea is practically constant, might indicate that the effect on the latter is on the proteid, but does not necessarily point that way, as it might merely be because the salts in the sap are not acted on at once.

I deemed it not worth while to test the action of some other poisons on Elodea, since, as table XXVIII shows, they all drive CO_2 from tap water.

I have been assuming that the cell sap contains salts like those in the tap water, from which CO_2 can be driven by the poisons applied. This cannot well be doubted; still it seemed desirable to prove it, if possible, by direct experiment. For this purpose I crushed a mass of Elodea from which I should say the CO_2 evolved at the temperature of the experiment could not possibly have been in excess of 0.060^{cc} N per hour. The sap was squeezed out and the material washed and squeezed again. Of course the plants were most thoroughly washed with distilled water before crushing. The sap was then diluted to 150^{cc}, and placed in the "control" bottle, while the

TABLE XXVIII.

May 2 and 3. The subject of each of these tests was 400^{cc} tap water.

Time	Experiment
2:36– 6:06	0.003
6:06 $\frac{M}{100}$ Co	Cl_2 in bottle.
6:06– 7:21	0.144
8:10– 9:16	0.000
9:16 $\frac{N}{100}$ H	Cl in bottle.
9:16–10:22	1.677
10:18–12:52	0.018
12:52 $\frac{M}{100}$ Pb(N	$O_3)_2$ in bottle.
12:52– 2:20	0.776
6:40– 8:20	0.003
8:20 $\frac{M}{1000}$ Cd(NO	$_3)_2$ in bottle.
8:20– 9:26	0.341

"experiment" bottle contained rather less Elodea, uninjured, in 150^{cc} distilled water. The sap was extracted and *filtered* during the evening, and evolved a great deal of CO_2 during the night. The record begins the next morning.

This shows an acceleration of the evolution of CO_2 from the sap, caused by the cadmium salt; also from the sound plants, but not quite as much during the one hour of the action of the cadmium.

TABLE XXIX.

May 23. "Respiration" by sap.

Time	Experiment	Control
9:40–1:22	0.040	0.261
A slight precipitate in the sap caused me to refilter it.		
1:40–6:34	0.046	0.155
6:34 $\frac{M}{1000}$ $Cd(NO_3)_2$ in both bottles.		
6:34–7:34	0.085	0.310

But the most remarkable thing about this experiment was the great evolution of CO_2 from the filtrate. The filtration seemed thorough both times, and after the second no precipitate could be detected before the cadmium was put in; afterward a very slight one was formed. The filtrate was colored and opalescent from the start. It is as impossible to treat the influence of cadmium on the evolution of CO_2 from the sap as depending on the carbonates in the latter as to suppose that the action of copper on the sound plants depends on the protoplasm. If there are processes going on in the sap which lead to the freeing of CO_2, the cadmium may very well act on these processes. By the duration and amount of this evolution the possibility of its being an inorganic reaction is precluded. It suggests very strikingly the accelerated respiration resulting from mechanical injuries, on which we are well informed by the work of Stich[45] and Richards.[46] But nothing hitherto known would indicate that there might be such a reaction by filtered sap. An identical performance observed by Hahn[47] in the sap of the spadix of

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[45] STICH, Die Athmung der Pflanzen bei verminderter Sauerstoffspannung und bei Verletzungen. Flora **74**: 1–57. 1891.

[46] H. M. RICHARDS, The respiration of wounded plants. Annals of Botany **10**: 531–582. 1896.

[47] M. HAHN, Chemische Vorgänge im zellfreien Gewebsaft von *Arum maculatum*. Ber. Deutsche Chem. Gesells. **33**: 3555–3560. 1900.

Arum did not suggest that the "respiration" might be abnormally vigorous, as the subject was chosen for its normally intense respiration. Hahn determined that a feature of the respiration by the Arum sap was the rapid consumption of sugar. His paper also discusses briefly the enzymes likely to be concerned. So far as can now be seen, we have here a performance which does not differ from respiration in the materials used, nor in the products, and is stimulated in the same way by mechanical violence and probably by poison, but every substance concerned in which passes through filter paper.

Enough of these tables on the action of the more fatal metallic poisons have been presented so that I anticipate that no other botanist will be attracted to the field as a promising one for conclusive work on respiration, or will imagine that a control of similar plants insures the validity of the results. The tap water experiment was a fortunate accident, where the utmost care would never have discovered the truth. It is very probable that the real respiration is accelerated by these poisons. This is indicated, for instance, in tables II and XV by the long continuance of the higher rate of respiration, and it is made more credible by the probably analogous action of K and Na still to be described.

The action of the poisons on the salts in the cell sap is not without interest in itself. The CO_2 comes ultimately from respiration; what effect freeing it may have on the respiration is problematical. In the other direction, it would seem that the reaction of the sap and the poison might protect the proteid, not of that cell, but of others. This can hardly hold good for as violent a poison as silver, but must of weaker ones, the trace of which remaining in solution would be harmless. Silver will kill Elodea in the presence of more than its equivalent of NaCl; yet I have found that tadpoles acclimated to rather strong NaCl and then thoroughly washed possess a limited immunity to silver poisoning.

There is a good general parallel between the toxicity of the various metals and their power to decompose carbonates, if that is a proper expression for the expulsion of CO_2 from the cell sap and tap water. This is conspicuously true of the three metals I have studied most thoroughly, Hg, Cu, and Zn; the more toxic the metal, the weaker the solution required to set free measureable CO_2. But this parallel is too hazardous to be dwelt upon. It appears also from the limited data at hand that the power to decompose carbonates is disposed to be parallel to the power to accelerate growth; for instance, Zn is very vigorous in proportion to its toxicity in both respects. The power to set free CO_2 is also proportional in general to the bleaching power of the different metals, as demonstrated by the solutions left with Elodea in them after the tests for toxicity.

Having found no strong metallic poison which did not drive CO_2 from the tap water, I wondered if other poisons might possess the same power. The first tried were antipyrin and iodin, both of which Jacobi found to accelerate respiration. I used such concentrations as he had done.

TABLE XXX.

May 12. Test of iodin. Experiment bottle contains Elodea in 400^{cc} "Neptune" water. Control is 400^{cc} tap water. The air after passing each runs through a small bottle containing 100^{cc} aq. dest. in which is more iodin than will dissolve.

Time	Experiment	Control
11:19-3:13	0.037	0.004
3:13 The iodin water	poured into the	other bottles.
3:13-6:13	0.068	0.002
6:13-7:43	0.060	0.000

TABLE XXXI.

May 10. Test of antipyrin. Experiment and control are same as in XXX.

Time	Experiment	Control
10:59-3:49	0.030	0.002
3:49 0.25 % an	tipyrin in both	bottles.
3:49- 5:59	0.047	0.002
5:59- 7:59	0.050	0.007
7:59- 9:32 (13.55^h)	0.047	0.005
9:32-11:32	0.045	0.000
11:58 $\frac{N}{100}$	HCl in both	bottles.
11:58- 2:22 (total C	O_2) 0.925	1.805

Both iodin and antipyrin accelerate the evolution of CO_2 from the Elodea, but not from the tap water. Their action must be on the the protoplasm. The acceleration observed was a shade greater than Jacobi's result with the same substances.

An experiment with potassium cyanid may be inserted here. The reason for performing it was the regard in which KCN is held by animal physiologists as a substance inhibiting respiration. The evidence for this rests, largely at least, on its preventing the consumption of oxygen, so that in KCN poisoning the blood retains its arterial color (its other effect in interfering with the carrying of the O by the blood being irrelevant here). I have made two tests of the evolution of CO_2 under the influence of KCN, one of which is reported here. Neither of them has the measure of quantitative exactness I could desire, but the apparent acceleration was so great it is hardly possible that it should have been an error.

TABLE XXXII.

January 22. Test of KCN. No control.

Time	Experiment
10:54–3:54	0.048
3:54 $\frac{N}{100}$ KCN	in bottle.
3:56–4:56	

The alkalinity of KCN prevented the escape of more than a trace of CO_2. To determine the CO_2 then the KCN solution was made slightly acid with H_2SO_4, and the CN precipitated with $AgNO_3$. It was then boiled.

Total CO_2 - - - 0.899^{cc} N.

As a control, 400^{cc} $\frac{N}{100}$ KCN (same solution) treated in same way. The CO_2 from it was - - - - - 0.208^{cc} N,

or for 500^{cc} - - - 0.260, leaving as the CO_2 from respiration in the experiment - - - - 0.639^{cc} N.

This result, with the evidence that oxidation is hindered by KCN, suggests forcibly that it is a substance which acts on "normal" but not on "intramolecular" respiration, which would amount to a proof for the already well supported theory that the evolution of CO_2 and the taking on of O are distinct activities of the plant; the latter, when it occurs, being dependent on the former, instead of *vice versa.* The action of KCN will well repay more study, whatever it may show, but I have had no time to follow it up.

The work on chemical stimulants was concluded with tests of some of the common mineral foods. Of these, calcium nitrate caused a more active evolution of CO_2 from Elodea, but as it was found to have the same effect on tap water, its action on the plant was of questionable nature, and the table showing its effect is omitted. Sodium chlorid and nitrate, and potassium chlorid, nitrate, sulfate, and iodid were all tested as to their action on tap water, from which none of them caused any escape of CO_2. To report the individual experiments showing this would be superfluous. Excepting a number of them which illustrate an altogether novel point in connection with the death of the plants, the individual tables of the results of the application of these salts can also be dispensed with, and their places will be taken by a single table showing the per cent. of acceleration of respiration. This per cent. is computed from what seems to me to be so much of the results of the experiments as represent the action of the salts on sound, unacclimated plants.

When any of these salts were applied to the plants in concentrations of $\frac{N}{20}$ to $\frac{N}{5}$ there was a prompt increase in the rate of respiration. This quickened rate remained constant in most of the experiments for a period of from twelve hours to more than a day; then it fell steadily or rose rather abruptly. The per cent. of acceleration is figured from the steady rate following the application of the salt, as compared with the preceding hours. The length of this period of uniformly accelerated respiration depended on the condition of the Elodea, on the nature of the salt and its concentration, and on the temperature, being shorter at higher temperatures.

The desired concentration of these salts was near enough to their limit of solubility so that applying them like the strong poisons would have introduced a possible source of large error in the CO_2 dissolved or soluble in the solution applied. This was avoided by the method employed with iodin (table XXX), letting the CO_2-laden air from each Elodea culture pass through another bottle containing the salt to be applied. Thus, if I had 2N KCl, and wished to test $\frac{N}{10}$, the Elodea was placed in 400^{cc} water, and the smaller bottle contained 22^{cc} of the $\frac{N}{10}$ solution, and water to 40^{cc}. The CO_2 content of the two bottles must have been practically the same when air had been passing through them over night.

TABLE XXXIII.

Salt	$\frac{N}{20}$	$\frac{N}{10}$	$\frac{N}{5}$
NaCl	29%	..	35%
$NaNO_3$	..	34%	58 (too high; cf. XXXV)
	..	..	32
KCl	41	42	58
	29.4	48.7	52
KNO_3	39	40.4	62
	36.4	..	61
K_2SO_4	..	31	..

A single comparison of KNO_3 and KI, both $\frac{N}{10}$, showed the same acceleration for eighteen hours at about 21°C., after which the KI material took a considerable lead and was in large part dead when the experiment ended.

It appears from this summary of results on K and Na, that the former is decidedly the stronger stimulant. Jacobi (p. 302) reports a much greater acceleration by $\frac{N}{20}$ NaCl than by $\frac{N}{20}$ KCl; but in six experiments, only part of which are represented in the above table, in which KCl or KNO_3 was in one bottle and the same concentration of the corresponding Na salt in the other, I have found no single instance in which the K salt failed to produce the greater effect. Kosiński (p. 141), at the other extreme, concludes that while KNO_3 and K_2SO_4 accelerate the respiration of Aspergillus, NaCl does not do so at all. My results on the respiration harmonize perfectly with the greater toxicity of K. True[48] rates the relative toxicity of K and Na to Spirogyra at 10:6. It seems to be about the same to Elodea.

It has been found by a number of observers that chlorids and nitrates of the same metals have the same toxicity, and the relatively few contrary statements do not bear the stamp of great probability. Coupin, for instance, rates the relative effectiveness of LiCl and $LiNO_3$ as 12:5, and that of $MnCl_2$ and $Mn(NO_3)_2$ as 1 : 13. Such a result is too surprising not to be doubted until there is more evidence for it. That chlorids and nitrates of the nutrient base often act differently in experiments of long duration, when used as fertilizers, is partly due to changes in the salts themselves. But working with salts whose base acted mildly enough so that concentrations even strong enough to cause plasmolysis could be used, it would have been possible to detect differences in the toxic or stimulating power of Cl or NO_3 ions too insignificant to suggest themselves at greater dilutions. Taken altogether, the NO_3 ion has been a shade the more active in my experiments; but there is no emphatic or constant difference in the stimulating action, in the effect on the turgor, or in the toxicity.

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[48] R. H. TRUE, The physiological action of certain plasmolysing agents. BOT. GAZ. **26**: 407–416. 1898.

Up to $\frac{M}{5}$, the stronger the salt, the greater the stimulation, but the added stimulation by no means remains proportional to the concentration; which is only another illustration of the general rule that the less of any food is present, the greater its efficiency in proportion to the amount.

I pass over any discussion of the probable mode of action of K and Na, whether as food or stimulant, because in the present state of our knowledge of the details of metabolism any such distinction in the case of essential food elements is difficult in words, and imaginary in practice.

The Elodea would always plasmolyze in 2 per cent. KNO_3 when the experiments began. In all the experiments then in which a salt was applied in the concentration of $\frac{N}{5}$ the cells must have been slightly plasmolyzed for a time. I was somewhat surprised that in such a case the extraction of water, as a purely physical process, did not operate to depress the respiration, but it seemed not to, as the acceleration was manifest from the time the salt was applied. When still stronger solutions are used, the physical action of extracting water and compressing the protoplast predominates over the chemical stimulation, and the respiration sinks below normal.

TABLE XXIV.

June 4. Test of $\frac{2N}{5}$ KNO_3 and KCl. Exp. is Elodea in 225^{cc} dist. H_2O. Small bottle has 150^{cc} $\frac{N}{1}$ KNO_3. Control Elodea in 225^{cc} dist. H_2O. Small bottle has 150^{cc} $\frac{N}{1}$ KCl.

Time	Experiment	Control
10:15– 6:09	0.073	0.070
6:09	Bottles poured together.	
6:09– 8:27	0.063	0.059
8:27–10:39	0.064	0.057
10:39– 7:21 (8.7) hrs.	0.044	0.045
7:21– 2:09	0.052	0.077
2:09–10:09	0.140	0.144

The low figures for the third period after applying the salt are partly due to a fall of 1.3° C. in temp.

The very rapid increase in the evolution of CO_2 occurred about as long after the application of the salt as was to have been expected with a solution but half as strong, from which it appears probable that the physical action of the salt has tended to protect the plant. The case is roughly analogous to those of the seeds of Lepidium[49] and the sporocarp of Marsilea,[50] which retain their vitality indefinitely in very strong alcohol, but succumb quickly if the alcohol contains water enough to soften the coats and make them permeable.

TABLE XXXV.

May 27. Test of $\frac{N}{5}$ $NaNO_3$.

Time	Experiment
6:58–11:52	0.033
11:52	Bottles poured together.
11:52–6:22	0.052
10:00 P.M.–10:00 A.M.	0.038
10:00–6:00 (8 hours.)	0.087
6:00 P.M., May 28	Tested as to turgor and found dead.
6:15–9:45	0.107

The exceptional acceleration of 58 per cent. by $NaNO_3$ (table XXXIII) comes from computing it from the first 6.5 hours of the salt's action. If 18 hours had been included it would have been only 30 per cent.

It remains to present a few of the experiments with K and Na fully enough to illustrate, as the last table does, the final rise of the curve of respiration, usually about a day after the application of the salt.

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[49] F. NOBBE, Handbuch der Samenkunde 116. Berlin. 1876.

[50] C. R. BARNES, Vitality of *Marsilea quadrifolia*. BOT. GAZ. **20**: 229. 1895.

TABLE XXXVI.

May 18. Test of $\frac{N}{5}$ KCl and NaCl.

Time	Exp., KCl	Cont., NaCl
9:55–2:35	0.055	0.054
2:35 Bottles	poured together.	
2:35–6:35	0.087	0.073
Record over	night not kept.	
8:36 A. M.–10:36	0.099	0.064
10:36–12:36	0.097	0.071
12:36– 2:36	0.109	0.076
10 P. M.	Almost dead.	Alive.
May 20, 11:00 A. M.	Dead.	Alive; not very unsound.

TABLE XXXVII.

May 20. Test of $\frac{N}{5}$ KNO_3 and $NaNO_3$. Subject, Ceratophyllum.

Time	Exp. KNO_3	Cont., $NaNO_3$
8:35–1:41	0.021	0.0296
1:41 Bottles	poured together.	
1:41–8:11	0.034	0.039
8:11–8:41 (12.5 hrs.)	0.057	0.058
8:41–11:41 (May 21)	0.073	0.053
11:41–11:53 (12.2 hrs.)	0.044	0.043

This experiment carried the plants past the point of most excessive combustion. The temperature during it was about 24.6°C.—the highest in any of these experiments.

TABLE XXXVIII.

June 2. Test of $\frac{N}{5}$ KNO_3 and KCl.

Time	Exp., KNO_3	Cont., KCl
8:37–12:43	0.028	0.027
12:43 Bottles	poured together.	
12:43–10:43	0.045	0.041
10:43–10:46 A. M.	0.067	0.074
10:46–9:46 (June 3)	0.068	0.078

The plants were "dead" at 10:46 A. M., June 3.

As already mentioned, plants treated with $\frac{N}{10}$ KI showed this phenomenon, but the parallel culture with KNO_3 did not while the experiment lasted.

This excessive evolution of CO_2 is undoubtedly a feature of the death of the plant. It is not easy to fix a time at which it begins, because the cells in a single leaf do not all die together ; but it is conspicuous while a very large part of the material is still evidently alive. It continues after the entire material, judged by its appearance under the microscope or by the test of plasmolysis, would be called dead. Two conceivable sources of this CO_2 suggest themselves; it might be allowed to escape by the removal of an obstruction when the protoplasm dies, as the acid in sour leaves gets an opportunity to destroy the chlorophyll ; or else its origin is in the protoplasm itself. As we have every reason to suppose, and none to doubt, that CO_2 diffuses readily through the living protoplasm, the first of these suggestions may be dismissed, and we may accept it as a fact that the escape of a large amount of CO_2 is not merely an accompaniment of death, but is a part of death.

If the phenomenon is thus fundamental in the demise of the plant, it must occur under other fatal conditions beside poisoning. In the experiments in which plants were boiled there was an amount of CO_2 evolved for which I was at the time quite unable to account. And I have had several opportunities to observe an increase of apparent respiration as Elodea succumbed to prolonged exposure to too high a temperature. Two of these follow; the first is an extract from the same experiment as table XI, showing the behavior of the control later in the day; during the hours included in table XI the control had been reasonably steady.

TABLE XXXIX.

December 10. Elodea at 39–41° C.

Time	Control
6:30– 8:05	0.087^{cc} N. per hr
8:05– 9:05	0.105
9:05–10:05	0.100
10:05–11:05	0.150
11:05–12:05	0.135
12:05–11:05 (11 hrs.)	0.143
11:05– 1:05	0.117

In the experiment recorded in table XL the rise in the control occurred in time to invalidate the results of the experiment culture.

While I have observed this feature of death only as a result of poisoning and of heat, it appears to me very possible that the immediate evolution of CO_2 when tubers were cut in Richards's work (*l. c.* 1896, p. 544) should be explained in the same way. CO_2 diffuses through cork so much more readily than does O that a great accumulation of it within the tuber, to be set free from any exposed surface, seems hardly natural. Kolkwitz[51] finds that grain ground to meal, soaked in alcohol, or heated to 100° C., all processes calculated to kill it, still gives off a very appreciable amount of CO_2. The behavior of Elodea sap might point in the same direction, though the consumption of large quantities of sugar, observed by Hahn in in the sap of Arum, demands a different interpretation.

TABLE XL.

December 6. Elodea at 39-41° C.

Time	Control
4:30- 5:30	0.070
5:30- 7:30	0.085
7:30- 9:30	0.097
9:30-11:30	0.110
11:30- 1:30	0.110

The evolution of CO_2, not only as a phase of death, but as a process continuing indefinitely after death, is another question on which I have sought for light. Reinke[52] has said that in the presence of atmospheric O dead seedlings and leaves continue to evolve CO_2 at ordinary temperatures, and his statement that no microorganisms are responsible for the phenomenon ought to be reliable. Schlösing[53] and Berthelot and André[54] have found CO_2 given off at high temperatures, 70–110° C., under the most various conditions. If these observations admitted of general repetition, all of our determinations of dry weights would be proven not to represent the entire solid matter of the plant.

My experiments with Hg, Cu, and Zn, which seemed at first to be the most striking proof of indefinite loss of CO_2 after death, can no longer be regarded as any positive evidence on the subject. The evolution from plants killed by K and Na decreased after they had been dead a few hours. The solubility of CO_2 in water makes my usual method of experimenting inappropriate to the determination of a time when CO_2 may cease to be evolved. I have tried one experiment which proved that if Elodea is left in water and kept at about 90° C. the evolution of CO_2 gradually diminishes, but at the end of four days is still as rapid as in the ordinary respiration of the same plants. As a control the same amount of material was placed in so little water that it became dry within twenty-four hours, and the evolution of CO_2 fell to a trace, without, however, quite ceasing. The material was again wetted and considerable CO_2 was evolved, though less than from the material which had been kept in water. It would appear from this that the usual dry-weight determinations are practically valid, and a number of tests which I have made with seeds show that at most a trace of CO_2 escapes as they dry at 100° C., or even a few degrees higher.

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[51] R. KOLKWITZ, Ueber die Athmung ruhender Samen. Ber. Deutsch. Bot. Gesells. **19**: 285–287. 1901.

[52] J. REINKE, Zur Kenntniss der Oxydationsvorgänge in der Pflanze. Ber. Deutsch. Bot. Gesells. **5**: 216–220. 1887.

[53] T. SCHLÖSING, Sur la combustion lente de certaines matières organiques. Comptes Rendus Acad. Sci. Paris **108**: 527. 1889.

[54] BERTHELOT ET G. ANDRÉ, Études zur la formation de l'acide carbonique et l'absorption de l'oxygéne par les feuilles détachées des plantes. Comptes Rendus Acad. Sci. Paris **118**: 45–54. 1894.

SUMMARY.

The method has been perfected until the results are reliable to one-fiftieth of a milligram.

No poison has been found *not* to act as a stimulant.

Metallic salts drive CO_2 from carbonates in the cell sap. This pseudo-respiration, under the action of strong poisons, is many times as active as the real respiration, and makes the study of the latter impossible.

CO_2 is given off from filtered sap squeezed from Elodea much more rapidly than from the Elodea before injury.

The stimulation by K salts is greater than that by Na salts, in about the proportion of their relative toxicity. No constant difference was found between chlorids and nitrates.

A considerable evolution of CO_2 is a feature of the breaking down of "protoplasm" into mere "proteid," in death.

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